with or without 20 μM PTU was determined. Next, we established mouse models with MPO-ANCA production. BALB/c mice were given intraperitoneal (i.p.) injection of PMA (50 ng at day 0 and day 7) and oral administration of PTU (5 mg/day) for 2 weeks. In this model, MPO-ANCA was produced by day 14 though an obvious vasculitic phenotype was not observed. These mice were divided into two groups, namely Group 1 with daily i.p. injection of Cl-amidine (0.3 mg/200 μl/day) (n=7) and Group 2 with daily i.p. injection of PBS (200 μl/day) (n=13). Two weeks later, serum MPO-ANCA titers and amounts of peritoneal NETs were compared between the 2 groups.

Results: In vitro NET formation induced by 20 nM PMA with or without 20 μM PTU was inhibited significantly by 200 μM Cl-amidine. Serum MPO-ANCA titers of Group 1 mice (32.3 ± 31.0 ng/ml) were significantly lower than those of Group 2 mice (132.1 ± 41.6 ng/ml). The amounts of peritoneal NETs in Group 1 mice was significantly smaller than those in Group 2 mice. These findings suggested that the NET formation was inhibited significantly by Cl-amidine both in vitro and in vivo, and that the MPO- ANCA production was also suppressed by Cl-amidine in vivo.

Conclusions: PAD4 inhibitor suppresses MPO-ANCA production through inhibition of NET formation in mouse model so that it could be a novel therapeutic modality for MPO-AAV in humans.

P16

INTERCELLULAR ADHESION MOLECULE-1 K469E(A/G) POLYMORPHISM AND ITS EFFECTS IN THE DEVELOPMENT OF DIABETIC NEPHROPATHY

Gu, H[1]
[1]Karolinska Institutet, Molecular Medicine and Surgery, Stockholm, Sweden

Introduction: Recent research has implicated that inflammation may be a key pathophysiological mechanism in diabetic nephropathy (DN), although its pathogenesis is multifactorial. Intercellular adhesion molecule 1 (ICAM-1) is an acute phase marker of inflammation and the ICAM-1 gene is located on chromosome 19p13.2 and resides a linkage region to diabetes and DN. To investigate whether ICAM-1 has effects in the development of DN, we have recently performed genetic and pathological studies of this molecule in Swedish and Malaysian subjects with normal glucose tolerance (NGT), diabetes and DN.

Methods: We genotyped six single nucleotide polymorphisms (SNPs) in the ICAM-1 gene with TaqMan allelic discrimination. We also determined plasma ICAM-1 levels with an enzyme-linked immune-sorbent assay kit.

Results: We found that non-synonymous SNP rs5498 (K469E A/G) was associated diabetes and DN and the G allele had a protective effect. Particularly, we found a high heterozygous index of this polymorphism presenting in both populations. The genotype distribution of this polymorphism was kept in Hardy-Weinberg Equilibrium and no duplicon in the genomic sequence was found. The ICAM-1 K469E(A/G) polymorphism resides in the 5th Ig-like domain of ICAM-1 protein. This domain is essential for dimerization, surface presentation and solubilisation of proteins of the protein and subsequently plays a crucial role in the activity of ICAM-1 protein in the interaction with LFA-1 and the adhesion of B cells. Furthermore, we found the carriers with heterozygous genotype had higher fasting glucose levels among newly diagnosed type 2 diabetes patients compared with the subjects with wild or mutant homozygous genotype. Plasma ICAM-1 levels were increased from the subjects with NGT, diabetes without DN to the patients with DN. Among diabetic patients with DN, the carriers with heterozygous genotype had higher plasma ICAM-1 levels compared with other patients.

Conclusions: Our study provided evidence that ICAM-1 has effects in the development of DN. The patients carrying with heterozygous genotype of SNP rs5498 (K469E A/G) in the ICAM-1 gene have higher risk susceptibility to DN. The combined approach with genotyping this polymorphism and measuring plasma ICAM-1 levels may be useful for prediction of DN in translation medicine.

P17

INTRAVASCULAR NEUTROPHIL EXTRACELLULAR TRAP (NET) RELEASE PROMOTE VASCULAR INJURY AND TUBULAR NECROSIS UPON ISCHEMIA/REPERFUSION INJURY (IRI) OF KIDNEY

Nakazawa, D[1], Kumar, S[1], Marschner, J[1], Anders, HJ[1]
[1]Medizinische Poliklinik- Klinikum Der Innenstadt, Nephrology, Munich, Germany

Introduction: Acute tubular necrosis (ATN) is common in severe acute kidney injury (AKI). Infiltrating neutrophils contribute to the crescendo of renal inflammation and kidney injury (necroinflammation), but how neutrophils contribute to ATN is not clear. We hypothesized that infiltrating neutrophils release neutrophil extracellular traps (NETs), which implies the release of cytotoxic DAMPs like histones that accelerate ATN and AKI.

Methods: In vivo; Postischemic AKI was induced in wild type mice by unilateral clamping of the renal pedicle for 35 minutes followed by reperfusion for 6h or 72h. Formation of NETs was identified by immunostaining using citrullinated histone 3 (CitH3) antibody. Intravascular NETs were confirmed by neutrophil elastase (NE)-DNA complex ELISA in plasma. Renalcell death was evaluated in kidney sections by Tunel staining and PicoGreen DNA assay of plasma. To investigate the effect of NETs inhibition in bilateral IRI (ischemic 35min, reperfusion 24h) group of mice were treated with either PAD inhibitor (PADin) or neutrophil depletion and sacrificed 24h after reperfusion. In vitro; To investigate whether hypoxia can directly induce NETs formation or indirectly via hypoxia-induced tubular cell death, 1) human neutrophils were incubated in 1% or 20%O2 for 24h, 2) the media of tubular cell line (TC), which were incubated in 1% or 20%O2 for 24h, and stimulated with neutrophils for 4h. Furthermore, PMA or histone- induced NETs were treated by PADin, anti-histone antibody and heparin. Histone-stimulated neutrophil media were applied to TC. NETs and TC injury in vitro were evaluated by CitH3 staining/MPO-DNA complex and LDH assay, respectively.

Results: IRI kidney showed increased positivity for CitH3 in areas of tubular necrosis of the outer medulla. Plasma levels of the NE-DNA complex were increased in a time-dependent manner as compared to sham-operated mice. NET-induced renal cell death was shown in terms of increased Tunel positive area in kidney and plasma DNA 6h after reperfusion compared to the sham group. Subsequently, NETs in plasma and kidney increased 15～24h after reperfusion. In bilateral IRI, treatment with both PADin and neutrophil depletion significantly reduced the plasma levels of NE-DNA complex and NETs area in kidney compared to the vehicle group. Treatment further improved renal excretory function in terms of reducing plasma creatinine levels. In vitro, the NET-